Identification of MHC restriction and anchor residues of Foot-and-Mouth Disease Virus derived bovine T cell epitopes

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Introduction

Foot-and-Mouth Disease Virus (FMDV) causes an extremely contagious disease in cloven hoofed animals with high morbidity. Although this disease can be controlled by the use of an inactivated whole virus vaccine, a number of disadvantages associated with its use and production have led to a cessation of FMDV vaccination in the EU and other countries with large livestock industries like USA, Canada and Australia.

This in turn has led to numerous research activities with the aim to develop alternative vaccination strategies. For the design of novel vaccines a thorough knowledge of antigenic regions recognized not only by B cells but also by T cells in the respective host species is essential. In the present study we aimed to identify T cell epitopes recognized by both cattle recognized by animal C129. Peptide 422 was the only one identified and found in both animals. In animal C129 three peptides caused a dose-dependent proliferative response in 3H-thymidine incorporation assays, the two overlapping peptides 252 and 253 and a third peptide with No. 422. PBMC from animal C129 responded only to one peptide, however this was again peptide 422.

Phenotype of proliferating peptide-specific PBMC

The phenotype of proliferating PBMC was investigated by CFSE assays. For both animals a proliferation of CD4+ T-helper cells was observed after stimulation with peptide 422, whereas cells with this phenotype did not respond to a control peptide (derived from Classical swine fever virus). This indicated that the observed response to peptide 422 was MHC class II restricted.

Identification of MHC class II anchor motifs by alanine scan

Alanine-substituted peptides of the original peptide 422 were synthesized and tested with PBMC from both cattle in CFSE proliferation assays. The frequency of proliferating CD4+ T cells was then determined as illustrated in contour-plots on the top, depicting results for the original peptide 422, a negative control peptide and alanine substituted peptide 422-5 (i.e. alanine residue was introduced at position 5). Diagrams below summarize results for the entire set of alanine-substituted peptides, indicating that in both animals the positions 5 and 9 were important for MHC binding. In the original peptide these positions were occupied by tyrosine residues (see one-letter code below the bar diagram, red circles). For animal C813 in addition positions 3, 4, and 7 seemed to be important for MHC binding (green circles).

Analysis of expressed MHC class II alleles and model of peptide-presenting MHC molecule

MHC class II alleles from both cattle were analyzed as follows: mRNA was isolated from PBMC, followed by RT-PCR with specific primers for bovine MHC class II loci DRB3, DQA and DQB. Subsequently PCR products were cloned and sequenced. Results are summarized in the adjoining table: for the DRB3 locus no common allele within both animals was found; however mRNA transcripts from DQA allele 22021 and DQB allele 1301 were found in PBMC from both animals.

Having identified common MHC binding motifs by alanine-scan within peptide 422 and common MHC class II alleles for DQA and DQB in both cattle we hypothesize that peptide 422 (representing aa-residues 22-36 of FMDV protein 1A) is presented in bovine MHC class II molecules encoded by DQA allele 22021 and DQB allele 1301. Furthermore, tyrosine residues at positions 5 and 9 (highlighted in red) seem to be important for binding of the peptide in the cleft of the MHC molecule.